Discrimination of alfalfa populations for resistance to *Aphanomyces euteiches* with real-time quantitative PCR

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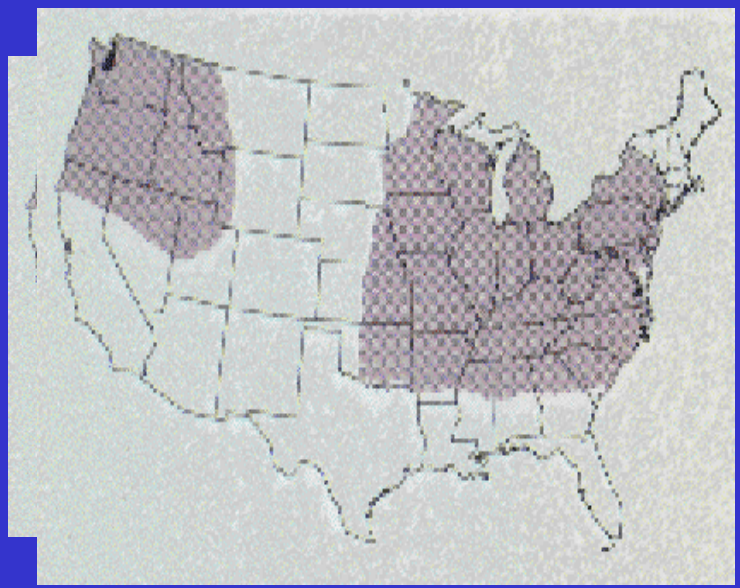
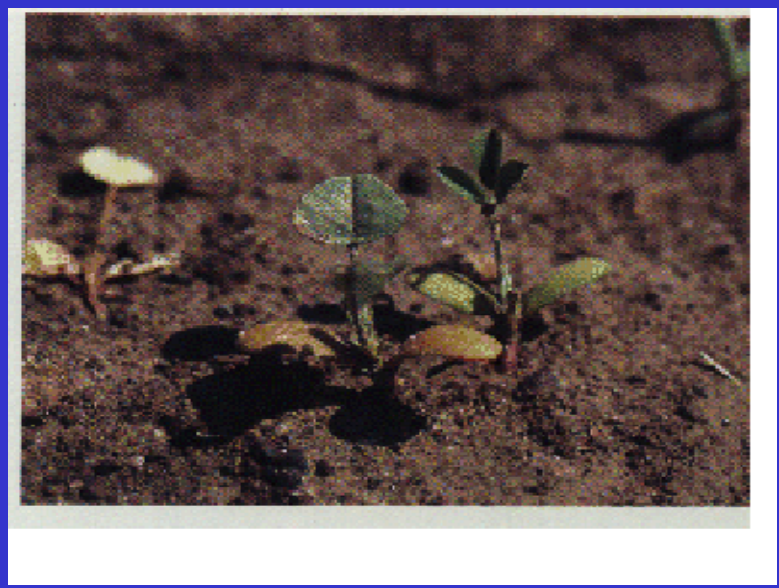
Aphanomyces euteiches

Plant pathogen that causes severe root rot disease in alfalfa, peas, and beans

Affected



Infected alfalfa



Aph^{res} Aph^{sus}

Rating[1] Alfalfa for Aphanomyces Resistance

1= healthy (R)

2= slight necrosis of roots and hypocotyl (R)

3 = moderate necrosis and stunting (S)

4 = extensive necrosis and stunting (S)

5= dead seedling (S)

[1]National Alfalfa Variety Review Board

Alfalfa Cultivar Classification[1] for Resistance to Aphanomyces Root Rot

- High Resistance = > 50% resistant plants
- Resistance = 31-50% resistant plants
- Moderate Resistance = 15-30% resistant plants
- Low Resistance = 6-14% resistant plants
- Susceptible = < 6% resistant plants

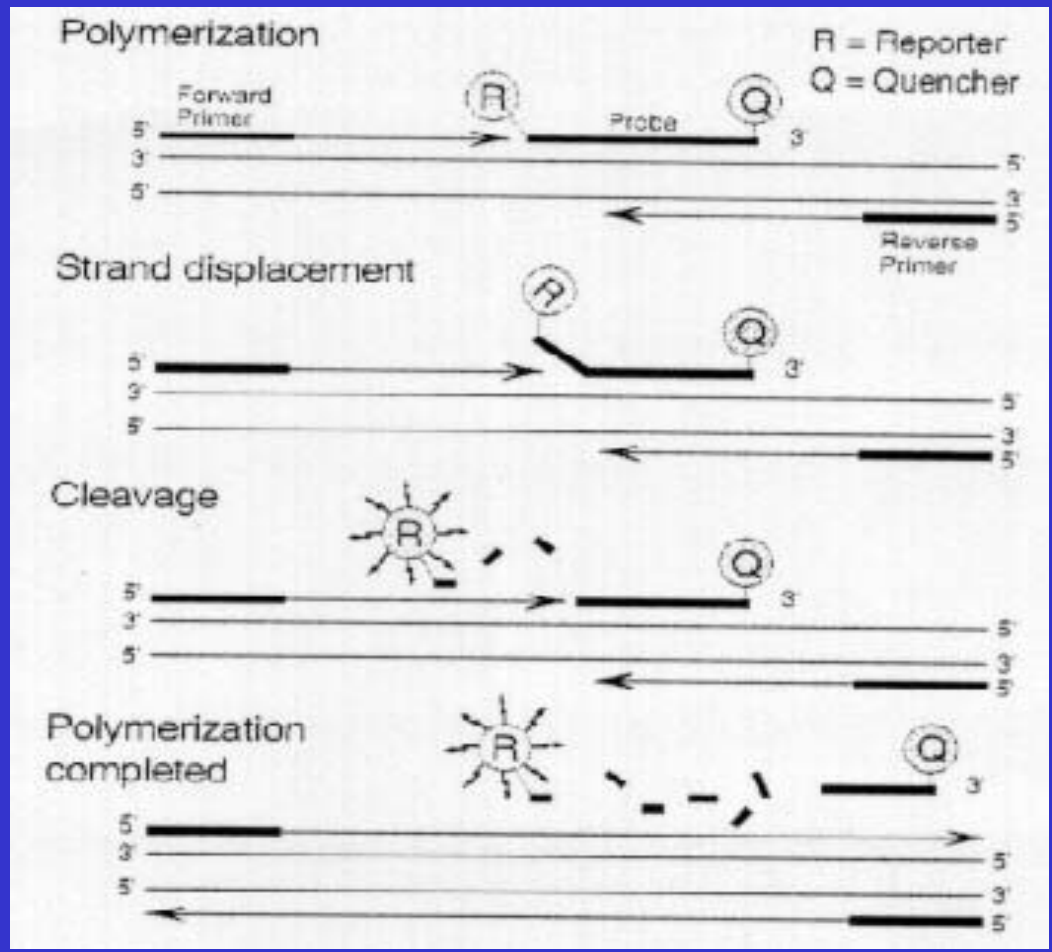
[1]National Alfalfa Variety Review Board

Limitations of accepted system for evaluation of resistance to *A. euteiches*

- Disease rating scale is subject to within and between-evaluator variation.
- Rating system has limited discriminatory power due to use of a semi-continuous scale.
- Over 200 plants must be individually scored for each variety.

Alternative: Use quantitative PCR to determine amount of pathogen in infected roots.

Separation of Reporter Dye and Quencher Dye Increases Fluorescence



OBJECTIVES

- Use quantitative PCR to investigate the relationship between resistance and quantity of *Aphanomyces* DNA in individual plants.
- Discriminate between resistant and susceptible check alfalfa populations using qPCR.
- Discriminate between commercial cultivars using qPCR.

MATERIALS and METHODS

Pathogen isolates

A. euteiches MF-1* (Race 1)

A. euteiches MW5 (Race 1)

A. euteiches NC 1* (Race 2)

A. euteiches WI-98 (Race 2)

* Type isolates for races 1 and 2

Alfalfa Check Varieties

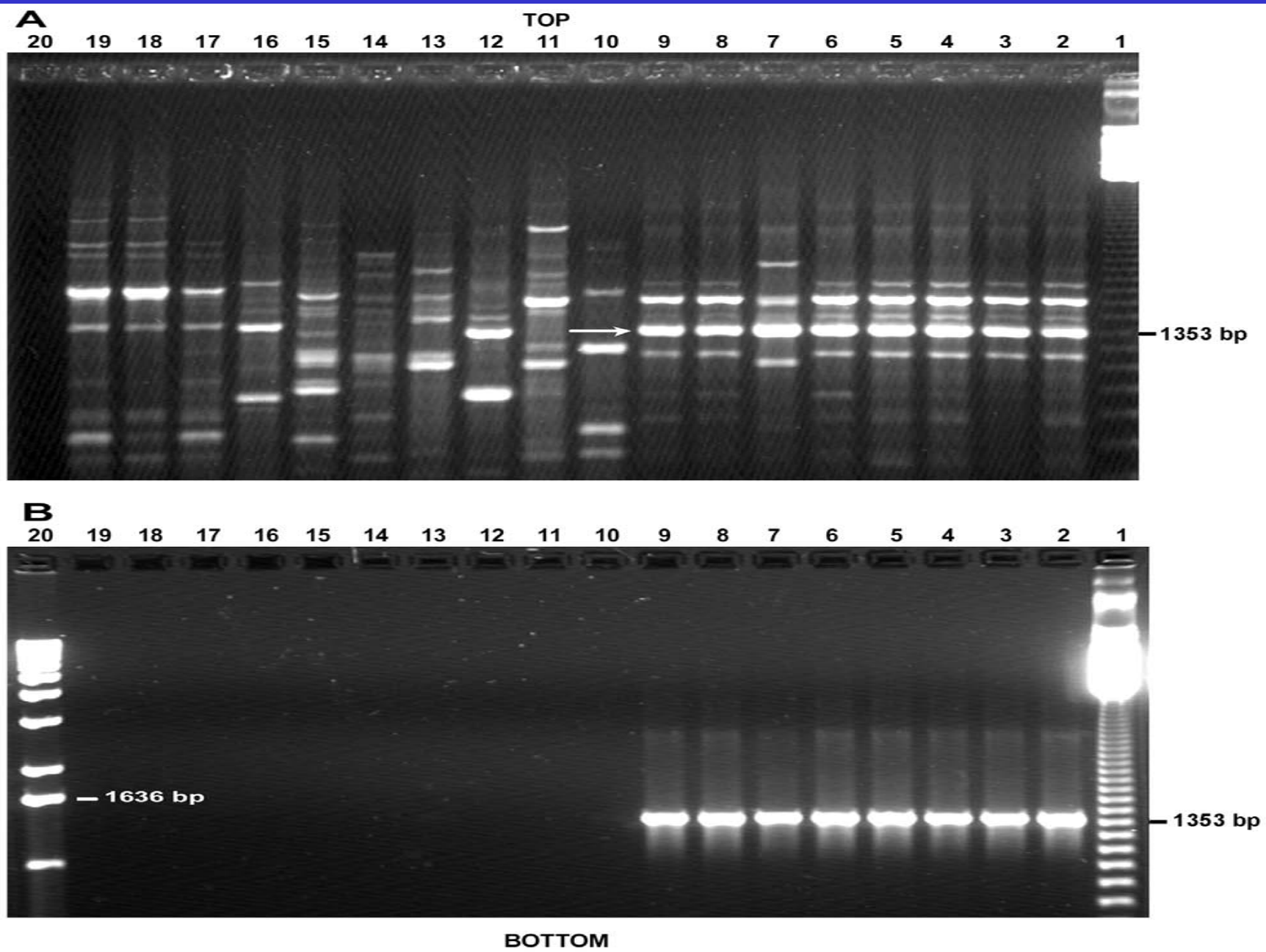
Race 1: Saranac (S), WAPH - 1 (R)

Race 2: Saranac (S), WAPH - 1 (S), WAPH - 5 (R)

Plant Inoculations

- NAAIC standardized test (1000 zoospores/plant).
- Plants were scored for DSI and individually harvested or randomly bulked (10 plants/bulk).
- DNA was extracted from roots.
- qPCR, with 3 repetitions DNA sample.

SCAR Specific for *A. euteiches*



Primer-probe Set for Quantitative PCR[1] of *A. euteiches*

100-TGCGACGCTGAGCTTGACCTTGTCGAATGCCTCTTGGAC

TGCAATGTCGTCCAAGACTTTGCAACCACCGAGCGAGCC

Forward Primer 136F

Taqman Probe

GCGCACTGCGTCGATCTCTTCATCTCAGCTTTGT-211

Reverse Primer 211R

[1]Amplifies a 76 bp fragment

RESULTS

RESULTS: Standard checks (disease free)

Saranac

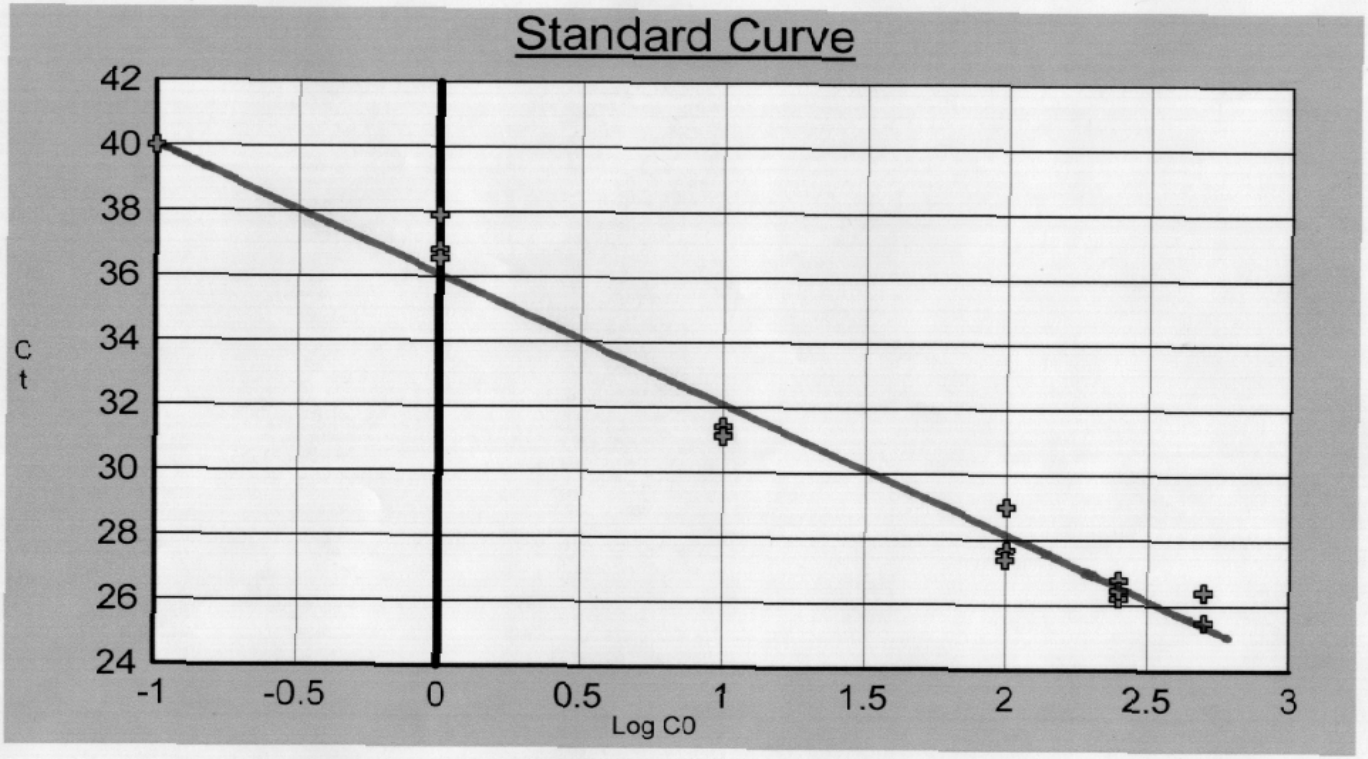
Waph-1

RESULTS: Standard checks (*A. euteiches* MF-1)

Saranac (S)

Waph-1 (R)

Detection of *A. euteiches* with Quantitative PCR

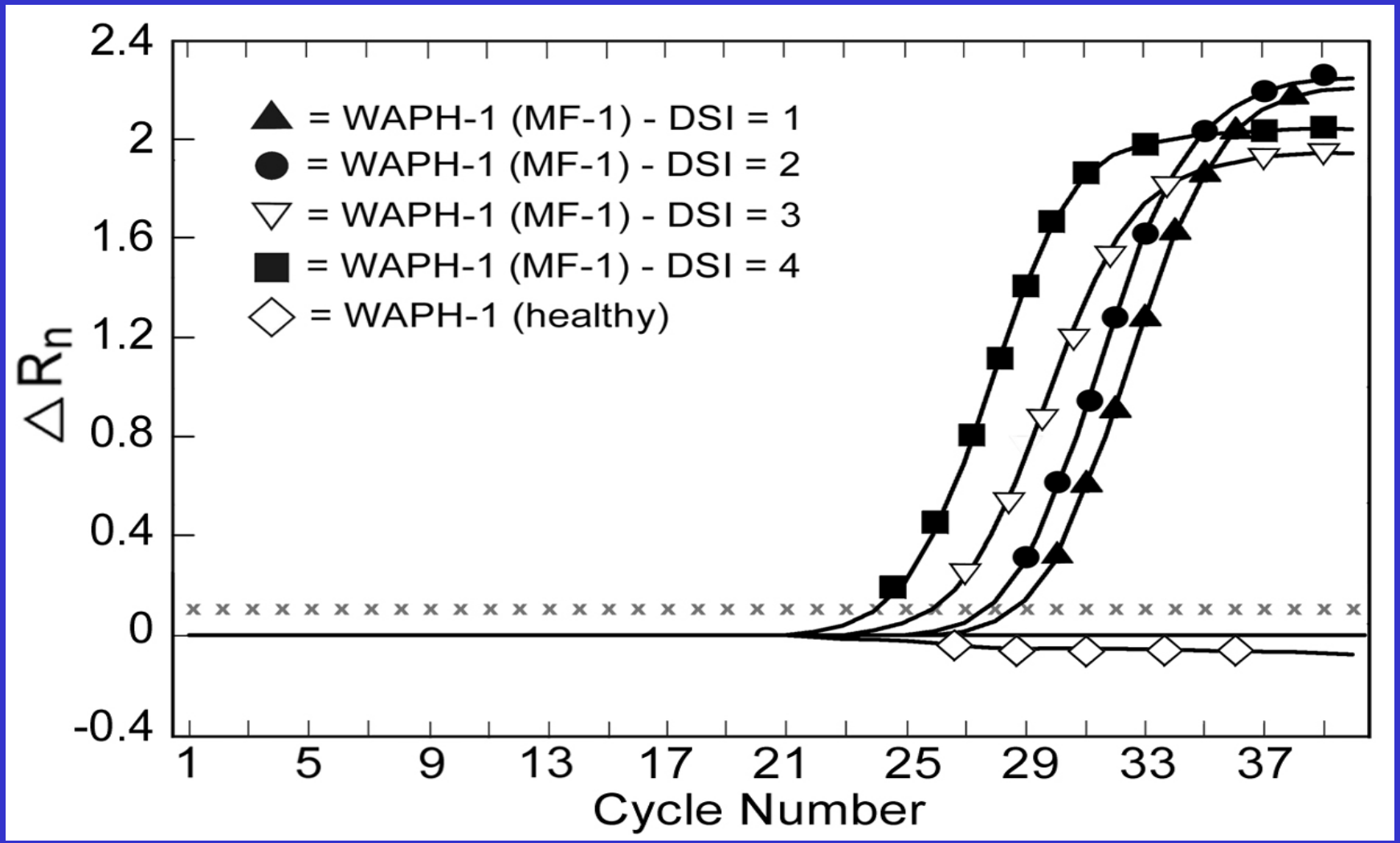


Slope: -3.972257

Intercept: 36.183788

Correlation: -0.989962

Detection of *A. euteiches* in Single Plants



Detection of *A. euteiches* in Single Plants

DSI	WAPH-1 (MF-1)	WAPH-5 (NC-1)
1	1.27 a	4.11 a
2	1.79 a	5.74 b
3	3.98 b	16.13 c
4	12.86 c	15.47 c
LSD ($\alpha = 0.05$)	1.97	1.46
ρ (Prob > $\lvert\rho\rvert$)	0.85 (<0.0001)	0.83 (<0.0001)

Bulk Analysis: Standard Check Populations

	A. euteiches MF-1		*A. euteiches* NC-1	
Population	ng DNA	DSI	ng DNA	DSI
WAPH-1	2.12a	2.79a	13.58a	3.99a
Saranac	8.75b	3.92b	12.66a	3.96a
WAPH-5	-	-	2.63b	2.66b
LSD(α=0.05)	1.60	0.13	1.23	0.13
$\rho(P > \lvert\rho\rvert)$	0.78 (0.0004)		0.79 (<0.0001)	

Bulk Analysis: Commercial Varieties

Variety	NAVRB Rating	ng DNA	DSI
WAPH-1	HR(✓)	1.08 (1)a	2.87 (3)ab
Winterking	R	2.25 (2)ab	2.70 (1)a
Ranier	HR	2.34 (3)ab	2.99 (6)b
WL 232HQ	HR	2.36 (4)ab	2.83 (2)ab
Ultralac	HR	2.71 (5) bc	2.88 (4)ab
WL 325HQ	R	3.72 (6)cd	2.98 (5)b
5246	MR	4.14 (8)d	3.29 (7)c
54V54	MR	4.66 (9)de	3.68 (12)d
Saranac	S(✓)	7.29 (17) f	3.84 (17)e
LSD($\alpha = 0.05$)		1.31	0.22
ρ ($P > \lvert\rho\rvert$)	0.54 (< 0.0001)		

Summary of Results

- PCR primer/probe set selectively amplified pathogen DNA and not host DNA.

- Quantification of DNA based on PCR assay was very precise ($R^2 \geq 0.97$).

Summary of Results

- Correlation between amount of pathogen DNA and disease severity was positive and highly significant for single plants and bulked plant samples.
- Separation of commercial varieties based on qPCR closely approximated published classification based on results of standard test.

Future Research Objectives

- Develop quantitative PCR assays for other alfalfa pathogens.
- Use qPCR assays to develop alfalfa germplasm with extreme resistance to multiple soilborne pathogens.
- Use qPCR assays to study population dynamics in plants infected with multiple pathogens.

Contributors

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